L3: Entry 1 of 2 File: USPT Oct 10, 1995

DOCUMENT-IDENTIFIER: US 5457041 A
TITLE: Needle array and method of introducing biological substances into living cells using the needle array

BSPR:
Expression of heterologous nucleic acids in various biological systems is a necessary tool in the study of gene regulation as well as a powerful technique in the development of agricultural varieties of plants, algae, fungi, and animals which possess improved traits. DNA or RNA may be introduced, for instance, into cells for transient gene expression wherein the introduced nucleic acids remain episomal and are not integrated into the replicating genetic material of the host cell. Transient expression of heterologous nucleic acids is valuable for testing the functional level of regulatory sequences in directing native RNA polymerases to precisely transcribe the gene of interest and polyadenylate the RNA transcript. Transient gene expression is also valuable to test the level and fidelity of sense or antisense transcription, translation and ultimately the functionality of the heterologous gene product.

BSPR:
Transgenic organisms, on the other hand, comprise plants and animals that have heterologous nucleic acid sequences artificially integrated as functional addendum to their natural genetic repertoire. Transgenic organisms also comprise plants and animals that have antisense heterologous nucleic acids integrated into their genetic repertoire to effect the attenuation of natural or artificial gene expression. See, e.g., U.S. Pat. No. 5,175,385 to Wagner et al.; U.S. Pat. No. 5,185,384 to Krimpenfort et al.; U.S. Pat. No. 5,175,383 to Leder et al.; U.S. Pat. No. 4,736,866 to Leder et al.; and U.S. Pat. No. 5,107,065 Anti-Sense Regulation of Gene Expression in Plant Cells. A successfully produced transgenic organism permanently contains the heterologous nucleic acid sequence stably integrated in a non-deleterious manner into its native genetic composition and is able to pass the corollary trait on to its natural progeny. Techniques are needed for efficient transformation of single cells, cells comprising tissues, and the production of transgenic organisms.

BSPR:
Functional nucleic acid sequences are introduced into living cells in various ways including well known methods using calcium phosphate and DEAE-dextran and polybrene mediated transfection, protoplast fusion, and introduction via cationic liposomes. Cells and tissues from different sources contrast sharply in their ability to take up and express exogenously added DNA and RNA. Irrespective of the method used to introduce nucleic acids into eucaryotic and procaryotic cells, the efficiency of transient or stable transformation and gene expression is determined largely by the cell and tissue type that is used.

BSPR:
More recently, plant tissue transformation has been produced through the use of particle-mediated "gene gun" technology. See, e.g. Sanford, The Biolistic Process TIB-TECH, 6:299-302 (1988); and U.S. Pat. No. 5,204,253 Method and Apparatus for Introducing Biological Substances into Living Cells. According to such "gene gun" technology, DNA or RNA is coated on micro spherical carrier particles of a dense metal, e.g., tungsten, gold or platinum. The carrier particles are accelerated to physically pierce and imbed within a living target tissue to carry nucleic acid into the tissue. A number of different mechanisms

have been employed to accelerate and project the coated carrier particles into target tissue including gunpowder ignition, pressured gas and a shock wave created by electric shock.

BSPR:
As in conventional "gene gun" technology where micro-particles are coated with biological substances, biological substances such as RNA and DNA may be deposited onto the needle array in a variety of ways including precipitation of the nucleic acid directly onto the structures, or by "dipping" in a non-toxic nucleic acid carrier suspension, for example a viscous substance such as glycerol or aqueous poly ethylene glycol, prior to delivery. The embodiment of the present invention comprising a cage-like structure at the needle tips provides enhanced carrying capabilities for holding of biological substances as well as capillary action for holding solutions or micro-particles coated with a biological substance for delivery to cell interiors.

BSPR:
In the method of delivery of biological substances using the needle array described herein, it is preferred that cell membrane perforations remain open for only a fraction of a second in order to minimize damage to the integrity of the cells comprising, for instance, meristem tissue. Natural lipid bilayer plasma membranes tend to reseal following minor non-traumatic disruption. Therefore a very rapid linear motion is preferred to effect puncture and withdrawal of the tip portions of the needle array from the cell during delivery of biological materials contained on the needle tips or on micro-particles carried by the needle tips. Nucleic acids DNA and RNA are extremely soluble in aqueous solutions such as the cytoplasmic contents of a living cell. Therefore, nucleic acids contained on the needle tips of the array or on the micro-particles carried by the needle tips quickly solubilize in the cell cytosol and trade adherence from the needle tips and micro-particles to the cytoplasm upon rapid linear withdrawal of the needle array. Alternatively, it may be desired to leave tips of the needles coated with the biological substances in the target cells for extended periods or even permanently. In such instances, at least the tip ends of the needles may be broken off immediately after insertion into the target cells as by the application of ultrasonic energy to, or lateral movement of, the needles followed by a rapid withdrawal of the balance of the needles from the cells. Further, such breaking of the needles may be enhanced by fabricating the needles with break away tip ends. Alternatively, the needles may be fabricated with detachable or releasably supported tip ends.

CLPV:
(b) mounting an array of micro-needles having tip portions carrying the biological substance or biological substance carrying micro-particles to a holder and aligning the array of needles with the tissue on the base, the needles having a length greater than the depth of the target cells and sufficient to pierce the target cells when the needles are forced into the tissue with the surface of a support substrate for the micro-needles adjacent to the surface of the tissue, and a spacing substantially equal to the width of the target cells to allow each needle to pierce one of the target cells;